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Relationship between quantity of IFNT estimated by IFN-stimulated gene expression in peripheral blood mononuclear cells and bovine embryonic mortality after AI or ET

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Abstract

Background: Interferon tau (IFNT), which is secreted into the uterine cavity during the maternal recognition period (MRP), is a key factor for establishment of pregnancy. The present study aims to clarify the relationship between the ability of a bovine conceptus to produce IFNT during the MRP and the conceptus's ability to establish pregnancy.

Methods: In the first experiment, IFNT (0, 500, or 1000 micrograms) was administered into the uterine horn ipsilateral to the CL 16 or 17 d after standing estrus, and mRNA levels of IFN-stimulated gene 15-kDa protein (*ISG15*) and *Mx2* in peripheral blood mononuclear cells (PBMCs) were determined. In the second experiment, we investigated *ISG15* mRNA expression in PBMCs during the MRP in cattle after either artificial insemination (AI) or embryo transfer (ET).

Results: Intrauterine administration of IFNT stimulated *ISG15* and *Mx2* gene expressions in PBMCs in cattle, and there was a positive correlation between the expressions of peripheral markers and the quantity of IFNT administered. In pregnant and normal interestrus interval (< 25 d) cattle (nEI cattle), expression levels of the *ISG15* gene showed similar patterns after AI and ET, and *ISG15* mRNA expression was increased in pregnant cattle but unchanged in nEI cattle. In contrast, *ISG15* gene expression in extended interestrus interval (greater than or equal to 25 d) cattle (eEI cattle) differed after ET compared with AI. In eEI cattle after ET, *ISG15* gene expression increased, such that the value on day 18 was intermediate between those of pregnant and nEI cattle. In eEI cattle after AI, *ISG15* gene expression did not increase throughout the observation period.

Conclusions: The results of the current study indicate that the quantity of conceptus-derived IFNT can be estimated by measuring *ISG15* mRNA levels in PBMCs from cattle. Using this approach, we demonstrate that *ISG15* gene expression during the MRP in eEI cattle differed after ET compared with AI. In addition, the modest increase in *ISG15* gene expression in eEI cattle after ET suggests that late embryo losses were due to delayed or insufficient growth of the conceptus during the MRP in cattle.

Keywords: Interferon tau, Interferon-stimulated gene 15-kDa protein, Embryonic mortality, Maternal recognition period, Cattle, Peripheral blood mononuclear cells, Corpus luteum, Estrous cycle

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Background

In most mammalian species, an embryo must signal its presence to the mother to establish a successful pregnancy. The signaling molecule in ruminants is interferon tau (IFNT), which is secreted from the trophoderm of the conceptus beginning at the blastocyst stage [1] and increases with elongation of the conceptus [2-5]. The production of IFNT peaks just before the conceptus attaches to the uterine epithelium (implantation) [6,7]; after implantation, the secretion of IFNT attenuates. IFNT interacts with its receptor on the uterine endometrium and modulates the secretion of prostaglandin $F_{2\alpha}$ from the endometrium, thereby maintaining the function of the corpus luteum (CL) [8-12]. Consequently, the IFNT level during the maternal recognition period (MRP) is considered to be important for successful establishment of pregnancy in ruminants [13-15].

For many years, IFNT had been thought to be secreted exclusively within the uterus and to not enter the peripheral circulation. However, some reports have suggested that the expression of various interferon-stimulated genes is upregulated in peripheral blood cells in pregnant cattle during the MRP [16-18]. Recent studies clearly demonstrate that a portion of the IFNT secreted into the uterine cavity enters the uterine vein and directly upregulates the expression of IFN-stimulated genes in both peripheral blood cells and the CL [19,20]. This upregulation of IFN-stimulated gene expression in peripheral blood is a potential target for new methods of early diagnosis of pregnancy. For example, mRNA levels of *Mx*, an IFN-stimulated gene, are increased in the peripheral blood mononuclear cells (PBMCs) of pregnant ewes from 15 through 30 d after insemination [17]. In addition, gene expression levels of *Mx1*, *Mx2*, and interferon-stimulated gene 15-kDa protein (*ISG15*) during the MRP are greater in pregnant compared with bred, nonpregnant cows [18]. A reliable method of measuring IFN-stimulated gene expression for early pregnancy detection potentially could be applied as early as 18 d after insemination of heifers [21].

In addition to its use for early pregnancy detection, a method measuring IFN-stimulated gene expression might be effective for evaluating embryo survival [16]. Embryonic mortality is a key factor causing pregnancy loss in cattle [22-26]. In classifying pregnancy loss according to luteolysis and interestrus intervals, a return to estrus before day 24 indicates 'early embryonic loss,' that occurring between days 24 to 50 is referred to as 'late embryonic loss,' and pregnancy loss detected after day 50 is characterized as 'fetal loss' [27]. Losses of pregnancy are characterized by early embryonic death, which occurs between days 8 and 16 of gestation [22,23]. Moreover, embryonic death in the case of late

embryonic loss has been suggested to occur beyond day 16 of gestation, because the lifespan of the CL is extended in this case [27]. Indeed, the expression levels of *ISG15* mRNA during the MRP differ between cattle that have undergone early compared with late embryonic loss after artificial insemination (AI) [16]. Most of the studies on embryo mortality involve cattle that have undergone AI, and the incidence and manner of pregnancy loss after embryo transfer (ET) might differ from those after AI, for which failure of fertilization is included in the calculation of pregnancy loss.

Therefore, we first sought to confirm the positive relationship between IFNT levels in the uterus and *ISGs* mRNA expression in the PBMCs of cattle. To demonstrate the relationship between production of IFNT by a bovine conceptus and embryonic mortality, we investigated *ISG15* mRNA expression in PBMCs during the MRP of cattle after either AI or ET.

Methods

Animals

All cattle were fed a grass silage-based diet *ad libitum*. All experimental procedures involving animals were approved by the committee for the Care and Use of Experimental Animals at the National Institute of Livestock and Grassland Science.

Experiment 1: Relationship between the quantity of IFNT administered into the uterus and the expressions of *ISG15* and *Mx2* mRNAs in PBMCs

Six female multiparous crossbred (Japanese Black \times Holstein-Friesian) beef cows weighing 610 to 730 kg were used in a randomized crossover design. Estrus was synchronized among these cows by providing each with two injections of prostaglandin $F_{2\alpha}$ (0.5 mg i.m.; cloprostenol, Nihon Zenyaku Kogyo, Fukushima, Japan) separated by an interval of 14 d.

Recombinant bovine IFNT (bTP-509A) produced by *E. coli*. [28] was diluted with PBS to a concentration of 0, 1, or 2 mg/ml; BSA (Nacalai Tesque, Kyoto, Japan) was added as needed to bring the total protein concentration of the IFNT-containing mixture to 2 mg/ml. Aliquots (500 μ l each) of the IFNT solutions were enclosed in plastic semen straws for administration into the uterine horn ipsilateral to the CL 16 or 17 d after standing estrus.

For determination of *ISG15* and *Mx2* mRNA expressions, blood samples (5 ml) were obtained at 2 h before and 0, 2, 4, 6, 8, 10, 12, 16, 20, and 24 h after administration of IFNT. Blood samples were collected through a 16-gauge jugular catheter (Unitika, Osaka, Japan) that had been implanted on the day before IFNT administration.

Experiment 2: *ISG15* gene expression during the MRP after AI or ET

Dried primiparous or multiparous cattle weighing 291 to 755 kg each either underwent AI at standing estrus (d 0) or received a frozen-thawed in-vivo-derived embryo into the uterine horn ipsilateral to the CL at 7 d after standing estrus. In order to compare the production of IFNT by a bovine conceptus among the pregnancy status, cattle after AI or ET were classified into 3 groups: pregnant cattle; extended interestrus interval (≥ 25 d) cattle (eIEI cattle); and normal interestrus interval (< 25 d) cattle (nIEI cattle) [29]. In this experiment, all eIEIs cattle (AI; $n = 9$, ET; $n = 11$) were used because the incidence of late embryonic loss was very low [27,30,31]. On the other hand, pregnant (AI; $n = 13$, ET; $n = 16$) and nIEIs (AI; $n = 19$, ET; $n = 17$) cattle were selected randomly. Untreated cycling cattle ($n = 15$) were used as controls.

Jugular blood samples were collected as described at 7, 16, 18, 21, and 25 d after standing estrus for analysis of *ISG15* mRNA expression and at 7, 12, 16, 18, 21, and 25 d after standing estrus for analysis of plasma progesterone (P_4) concentrations. Pregnancy was diagnosed by transrectal ultrasonography at 30 to 50 d after standing estrus.

Embryos

Embryos were collected nonsurgically from superovulated Japanese Black cattle on day 7 after standing estrus, as described previously [32]. Embryos staged at grade 1 or 2 blastocysts according to the IETS manual [33] were cryopreserved by using ethylene glycol as a cryoprotectant [34]. After being thawed, the embryos were cultured in TCM199 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS (ICN Biomedicals, Aurora, OH, USA) at 38.5°C in 5% CO₂, 5% O₂, 90% N₂. After 6 h of culture, the quality of viable embryos was reevaluated and only grade 1 or 2 embryos were used for ET.

Blood sample processing

Blood samples for the determination of plasma P_4 concentrations were collected into heparin-containing tubes (Terumo, Tokyo, Japan), and plasma was separated by centrifugation at 4°C for 30 min and stored at -30°C until assayed. Blood samples for RNA extraction were collected into tubes containing potassium EDTA (Terumo). Samples were centrifuged at 4°C for 30 min, and buffy coat fractions were collected. Contaminating red blood cells were removed by hemolysis, and PBMCs were isolated. The PBMCs were resuspended in TRIzol reagent (Invitrogen) and stored at -80°C until analysis.

RNA extraction, cDNA synthesis, and quantitative real-time RT-PCR

Total RNA was extracted from PBMC samples by using TRIzol (Invitrogen) in accordance with the manufacturer's

protocol. Single-stranded cDNA was synthesized from 1 µg of RNA by using SuperScript II Reverse Transcriptase (Invitrogen) with oligo-dT₁₂₋₁₈ primer (Invitrogen) in accordance with the manufacturer's instructions. The resulting cDNA was used as a template for quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis. The sequences of primers and probes for *ISG15*, *Mx2* and *GAPDH* are listed in Table 1. Each reaction mixture consisted of cDNA, forward and reverse primers (200 to 500 nM each), probes (100 to 300 nM each), Brilliant II QPCR Master Mix (Agilent Technologies, Palo Alto, CA, USA), and nuclease-free water in a total reaction volume of 20 µl. Thermocycling conditions included initial sample incubation at 50°C for 2 min, then 95°C for 10 min, followed by 50 cycles of 30 sec at 95°C followed by 90 sec at 60°C. Serial dilutions of plasmid containing *ISG15*, *Mx2* or *GAPDH* were used as standards. Results are reported as the n -fold difference relative to a calibrator cDNA (i.e., experiment 1: transcript of sample at 2 h before IFNT administration; experiment 2: transcript of sample on day 7 after standing estrus) after normalization of the transcript signals to the endogenous control *GAPDH*.

P_4 assay

Plasma P_4 concentrations were determined by using a double-antibody enzyme immunoassay as described previously [35]. Briefly, 100 µl of plasma sample was extracted twice with 2 ml diethyl ether in a glass tube. After evaporation of the diethyl ether, the residue was dissolved in 500 µl of assay buffer (0.01 M PBS, pH 7.0, containing 1% [w/v] BSA) and used for the assay. Microtiter plates were coated with anti rabbit γ globulin goat serum (50 ng/well in 0.1 M carbonate buffer, pH 9.6; Cappel, MP Biomedicals, Solon, Ohio, USA) as the priming antiserum. A rabbit antiserum raised against 11 α -hydroxy-progesterone-HS-BSA (a gift from Dr. Takenouchi) was diluted to 1:800,000. Progesterone-3-CMO-HRP (Cosmo Bio, Tokyo, Japan) diluted to 1:500,000 was used as the steroid-enzyme conjugate for visualization of the signal. Assay sensitivity was 0.1 ng/ml for 100-µl plasma samples; therefore, a sample yielding a signal below this threshold was assigned a value of 0.1 ng/ml. The intra- and interassay coefficients of variation were 7.8% at 4.8 ng/ml and 2.8% at 5.0 ng/ml, respectively.

Statistical analysis

Statistically significant differences ($P < 0.05$) in relative *ISG15* and *Mx2* mRNA expression levels were determined by using one-way ANOVA for repeated measures followed by Dunnett's test. Regression analysis was used to evaluate the relationship between the levels of relative *ISG15* and *Mx2* mRNA expressions at 4 h after administration of

Table 1 Oligonucleotide primers and probes used for real-time RT-PCR analysis

Gene	GenBank accession no.		Sequence	Position (nucleotide no.)
ISG15	NM_174366	Forward primer	5' GGGACCTGACGGTGAAGATG 3'	67-86
		Reverse primer	5' GAAAGCAGGCACATTGATCTTCT 3'	160-182
		TaqMan probe	5' TCCTGGTGCCTCTGAGGGACTCCAT 3'	103-127
Mx2	NM_173941	Forward primer	5' AAATCACCTACCGCAACATTACG 3'	772-794
		Reverse primer	5' GCCAAGTCCATTCCAGCTA 3'	853-872
		TaqMan probe	5' ATGTTCTGGGCTCTCCGA 3'	833-850
GAPDH	U85042	Forward primer	5' GGCACAGTCAAGGCAGAGAAC 3'	238-258
		Reverse primer	5' GGATCTCGTCTCTGGAAGATG 3'	291-311
		TaqMan probe	5' CATCAATGGAAAGGCCA 3'	270-286

IFNT and the dose ($\mu\text{g}/\text{kg}$ body weight) of IFNT administered into the uterus.

The estrus was decided by standing behavior or sharp decline in plasma P_4 values. In addition, untreated cycling cattle were used as controls. Statistically significant differences ($P < 0.05$) in relative *ISG15* mRNA expression and plasma P_4 concentrations on days 16, 18, and 21 were determined by using one-way ANOVA followed by the Tukey HSD test within each day, and those on day 25 were determined by using Student's *t* test. Mean estrous cycle length in eIEI cattle were analyzed by using Student's *t* test.

Results

Relationship between the quantity of IFNT administered into the uterus and the *ISG15* and *Mx2* mRNA expression levels in PBMCs

Intrauterine administration of IFNT significantly ($P < 0.05$) increased *ISG15* and *Mx2* mRNA expressions in the PBMCs of cattle relative to pretreatment values. *ISG15* (Figure 1A) and *Mx2* (Figure 1C) mRNA levels began to increase at 2 h after administration of IFNT, peaked at 4 h, and subsequently decreased and returned to the basal. The *ISG15* ($r = 0.88$, $P < 0.01$) and *Mx2* ($r = 0.83$, $P < 0.01$) mRNA expression levels at 4 h after IFNT treatment were positively correlated with the dose of IFNT administered into the uterus (Figure 1B and 1D).

ISG15 mRNA expression during the MRP after AI or ET

Among cattle that underwent AI, the average *ISG15* mRNA expression in PBMCs on days 18, 21, and 25 was significantly ($P < 0.05$) higher in pregnant cattle ($n = 13$) than in any other group on the same day (Figure 2A). *ISG15* mRNA expression on days 18 and 21 after AI did not differ among eIEI ($n = 9$), nIEI ($n = 19$), and cycling control cattle ($n = 15$).

Among cattle that underwent ET, the mean *ISG15* mRNA on day 18 was significantly ($P < 0.05$) higher in pregnant cattle ($n = 16$) than in nIEI ($n = 17$) and cycling control cattle ($n = 15$) (Figure 2B). *ISG15* mRNA

expression on day 18 after ET in eIEI cattle ($n = 11$) was intermediate between the value for pregnant cattle and both of those for nIEI and cycling control cattle, although none of these differences reached significance.

Changes in plasma P_4 concentrations after AI or ET

After AI or ET, plasma P_4 concentration on days 7, 12, 16, and 25 did not differ between pregnant and eIEI cattle (Figure 3). On day 18 after AI, nIEI cattle had significantly lower plasma P_4 concentrations than pregnant cattle, and numerically but not significantly lower concentrations than eIEI and control cattle. On day 18 after ET, plasma P_4 concentrations in nIEI cattle were significantly lower than those in both pregnant and eIEI cattle. Furthermore, plasma P_4 concentration on day 21 after AI or ET was significantly ($P < 0.05$) lower in nIEI and control cattle than in pregnant and eIEI cattle.

Return to estrus in eIEI cattle after AI or ET

In eIEI cattle after AI and ET, the mean length of the estrous cycle was 35.1 ± 3.8 and 39.5 ± 1.8 d, respectively (Table 2); these values did not differ significantly. The distribution of return to estrus in eIEI cattle was 25 to 29 d (44.4% of the group) for AI and 40 to 44 d (36.4%) for ET.

Discussion

Although IFNT is a key factor for the first step of establishment of pregnancy, whether the ability of a bovine embryo to produce IFNT reflects its capacity to establish pregnancy has not been clarified. Measuring the expression of IFN-stimulated genes has been suggested as a possible method for assessing embryo survival as well as for early detection of pregnancy [16]. However, the correlation between the quantity of IFNT secretion excreted into the uterus and the levels of IFN-stimulated gene expression in PBMCs had not been determined previously. Therefore, we measured the levels of *ISG15* and *Mx2* mRNAs in bovine PBMCs after intrauterine administration of recombinant IFNT. Intrauterine administration of IFNT stimulated *ISG15* and *Mx2* gene expressions in PBMCs in

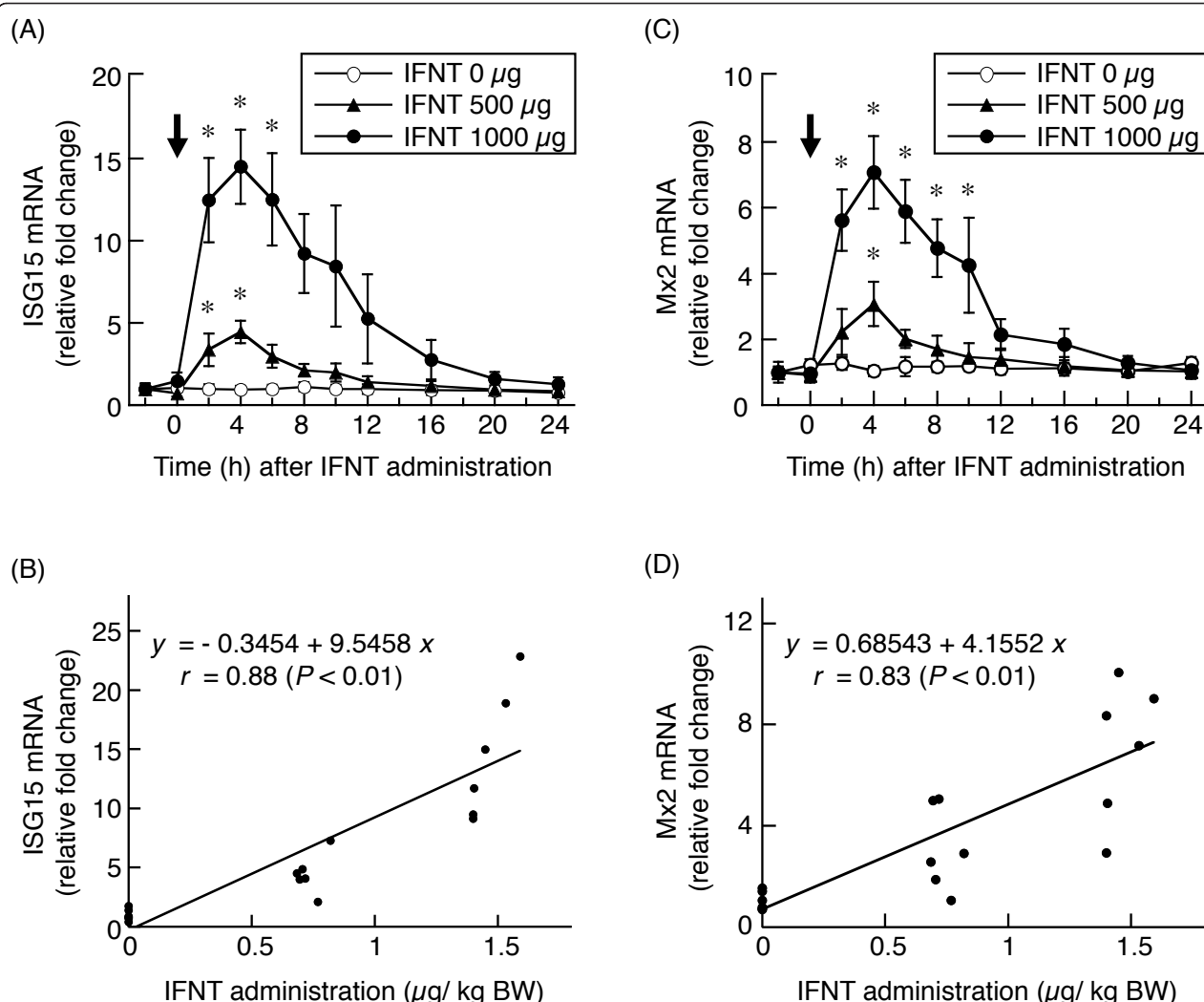


Figure 1 *ISG15* and *Mx2* mRNA expressions after intrauterine administration of IFNT. *ISG15* (A) and *Mx2* (C) mRNA expressions (mean \pm S.E.M.) in PBMCs of cattle after intrauterine administration of IFNT (arrow; 0, 500, or 1000 µg); data are presented as relative fold change from the amount at 2 h before IFNT administration. *, $P < 0.05$ compared with value at 2 h before IFNT administration. Regression line of *ISG15* (B) and *Mx2* (D) mRNA expressions at 4 h after IFNT administration and concentration of IFNT administered into the uterus.

cattle, and there were positive correlations between the expressions of peripheral markers and the quantity of IFNT administered. In addition, the correlation coefficient between *Mx2* and IFNT ($r = 0.83$) was slightly lower than that between *ISG15* and IFNT ($r = 0.88$). These results suggest that the quantity of conceptus-produced IFNT could be monitored by measuring *ISG15* and *Mx2* mRNA expressions in PBMCs, thereby providing a powerful non-invasive tool to evaluate embryonic mortality.

The present study is the first to report changes in *ISG15* mRNA expression in PBMCs in cattle after ET. In pregnant and nIEI cattle, *ISG15* gene expression after ET followed a similar pattern as the expression after AI. In the current study, *ISG15* mRNA expression in pregnant cattle was increased during the MRP as reported

previously [18,21]. In cattle, a portion of the IFNT secreted by the developing conceptus during the MRP enters into the uterine vein and directly upregulates the expression of IFN-stimulated genes in peripheral blood cells [20]. The present study revealed that the amount of IFNT administered into the uterus is positively correlated with *ISG15* mRNA expression in PBMCs. In addition, some studies have demonstrated that the quantity of IFNT production parallels the degree of trophoblastic elongation in cattle [2-4] and sheep [5]. Therefore, the magnitude of increase in *ISG15* mRNA expression in PBMCs likely reflects the amounts of conceptus elongation and IFNT secretion.

nIEI cattle lacked any increase in *ISG15* mRNA expression during the MRP. Previous investigations

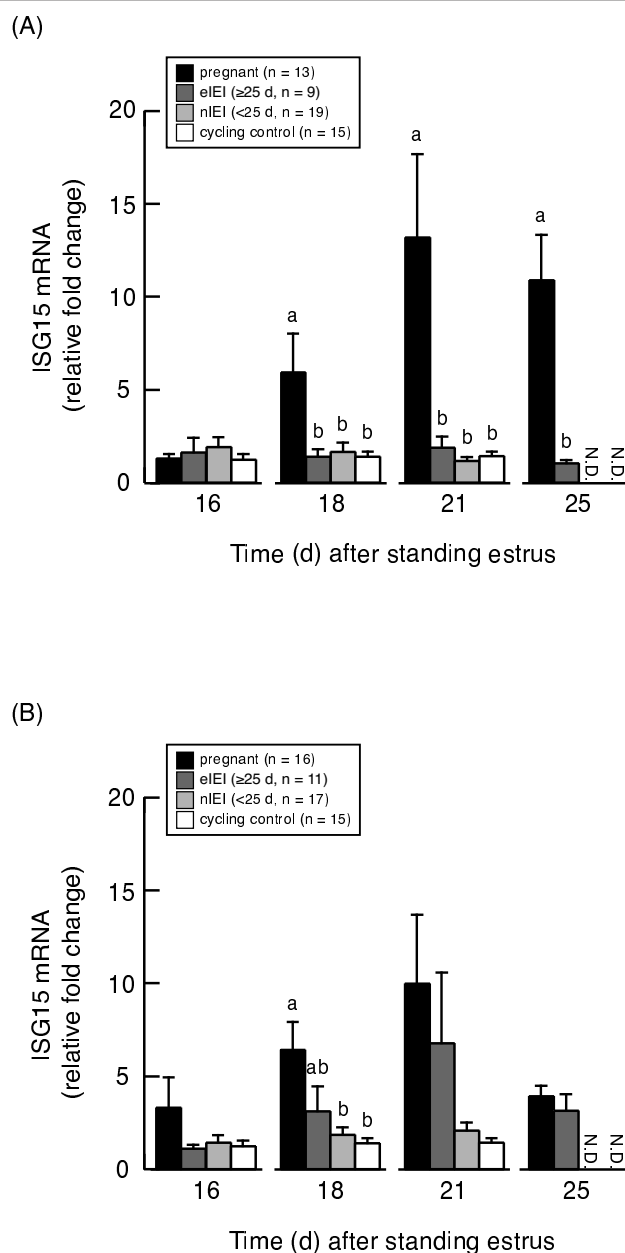


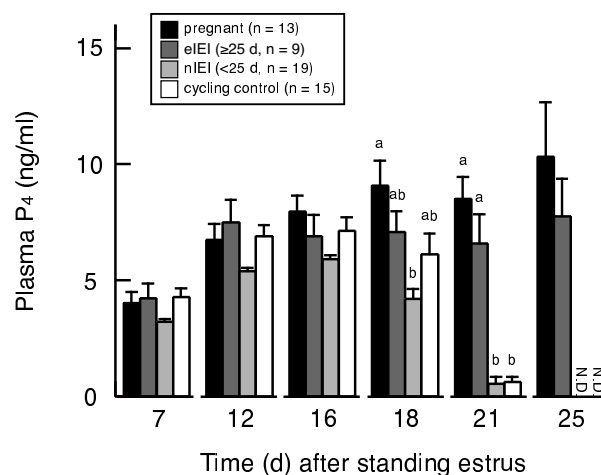
Figure 2 *ISG15* mRNA expressions in each pregnancy status of cattle after AI or ET. *ISG15* mRNA expression (mean ± S.E.M.) on days 16, 18, 21, and 25 after standing estrus in pregnant, extended interestrus interval (≥ 25 d, eIEI), and normal interestrus interval (< 25 d, nIEI) cattle after AI (A) or ET (B). Data are presented as relative fold change from value on day 7; comparisons were made within each day. Different letters indicate a significant ($P < 0.05$) difference between values.

[22,36] indicate that fertilization rates are high (on the order of 90%) in cattle, suggesting that embryonic death is responsible for the majority of reproductive failure. Moreover, the greatest incidence of embryonic death is considered to occur between days 8 and 16 after insemination [22]. These previous findings might well explain the results of the current study, such that early

embryonic loss (that is, before the MRP) would account for the observed lack of increase in *ISG15* gene expression in nIEI cattle.

Unlike those in pregnant and nIEI cattle, the changes in *ISG15* gene expression in eIEI cattle differed depending on whether the animal underwent AI or ET. In eIEI cattle after ET, *ISG15* gene expression increased

(A)



(B)

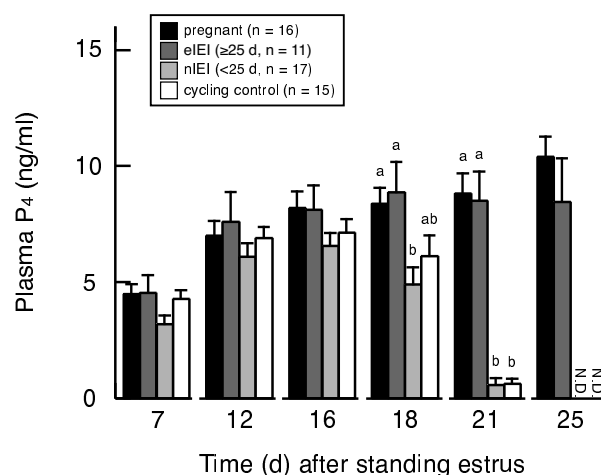


Figure 3 Plasma progesterone concentrations in each pregnancy status of cattle after AI or ET. Plasma progesterone concentration (mean ± S.E.M.) on days 7, 12, 16, 18, 21, and 25 after standing estrus in pregnant, extended interestrus interval (≥ 25 d, eIEI), and normal interestrus interval (< 25 d, nIEI) cattle after AI (A) or ET (B). Comparisons were made within each day. Different letters indicate a significant ($P < 0.05$) difference between values.

Table 2 Estrous cycle length in eIEI cattle after AI or ET

	Group mean ± SEM	Estrous cycle length (d) ^a					
		25-29	30-34	35-39	40-44	45-49	50-
AI (n = 9)	35.1 ± 3.8	44.4% (4)	11.1% (1)	22.2% (2)	0% (0)	11.1% (1)	11.1% (1)
ET (n = 11)	39.5 ± 1.8	9.1% (1)	18.2% (2)	27.3% (3)	36.4% (4)	9.1% (1)	0% (0)

^aValues are given as % of group (n).

somewhat, with the value on day 18 intermediate to those of pregnant and nIEI cattle. This result suggests that even if the conceptus can secrete IFNT during the MRP, pregnancy is not always established. As mentioned earlier, the *ISG15* mRNA expression in PBMCs reflects not only the quantity of conceptus-produced IFNT but also the degree of conceptus growth. Therefore, it is possible that the comparatively limited increase in *ISG15* gene expression in eIEI cattle results from delayed conceptus growth, as previously suggested to occur in dairy cows [16]. As another possibility, the comparatively low IFNT production in eIEI cattle suggests insufficient growth and low viability of conceptuses, which consequently die beyond the MRP. In addition, because IFNT acts on the endometrium to regulate genes important for uterine receptivity and conceptus growth [37-39], the comparatively low IFNT quantity in eIEI cattle might be insufficient to induce endometrial functions necessary for conceptus growth.

Moreover, in eIEI cattle after ET, there was no correlation between *ISG15* mRNA expression and plasma P₄ on the same day (data not shown). In addition, plasma P₄ concentrations during the MRP after ET were not different between eIEI and pregnant cattle, indicating that there was no difference on the CL function. Taken together, these findings suggest that CL regression is inhibited regardless of the quantity of IFNT. Further study is needed to clarify the mechanism of beginning of CL regression in eIEI cattle after ET.

Since *ISG15* responds to not only various types of IFNs but also viral infection [40,41], increase of *ISG15* gene expression found in this study might attribute to viral infection. Moreover, it was reported that uterine infections in cows can extend IEs independent of pregnancy status [42,43]. Therefore, it could not deny the possibility that the elevation of *ISG15* gene expression was induced by the subclinical uterine infection in eIEI cattle after ET, although the clinical uterine infection was not detected in the present study. The effect of the subclinical uterine infection on the IEs and *ISG15* gene expression must be investigated in future.

In contrast to the scenario after ET, *ISG15* gene expression did not increase during the MRP of eIEI cattle after AI, and the values did not differ compared with those in nIEI and cycling control cattle. The reason for the invariable level of *ISG15* gene expression is unclear, but perhaps our method for measuring *ISG15* mRNA expression in PBMCs was insufficiently sensitive to detect slight increases. In addition, plasma P₄ concentrations did not differ between eIEI and pregnant cattle after AI. These results suggest that CL function was maintained during the MRP in eIEI cattle after AI and that estrous cycle length was extended even though embryonic loss occurred before the MRP. Although it

was not clarified how estrous cycle length was extended in eIEI cattle after AI, we speculate that embryonic death in eIEI cattle after AI occurred somewhat later than in nIEI cattle after AI. In the present study, the most frequent range of return to estrus in eIEI cattle after AI was 25 to 29 d (44.4%). This slightly extended interestrous period in many eIEI cattle after AI supports our speculation that later embryonic death in eIEI than nIEI cattle is associated with inhibition of CL regression and delay of return to estrus. Moreover, regardless of breeding method, the number of days until return to estrus in eIEI cattle was not correlated with *ISG15* mRNA level during the MRP (data not shown). Accordingly, the distribution of estrous cycle length might be influenced by the stage of follicular development at the beginning of CL regression rather than by the quantity of IFNT secreted by the conceptus.

Conclusion

The results of the present study indicate that the quantity of conceptus-derived IFNT in cattle can be estimated by measuring *ISG15* gene expression in PBMCs. Using this approach, we demonstrate that *ISG15* gene expression during the MRP in eIEI cattle differed depending on whether cows underwent AI or ET. In addition, the slight increase in *ISG15* gene expression in eIEI cattle after ET suggests that late embryonic losses were due to delayed or insufficient conceptus growth during the MRP.

Abbreviations

AI: Artificial insemination; CL: Corpus luteum; ET: Embryo transfer; IFNT: Interferon tau; *ISG15*: Interferon-stimulated gene 15-kDa protein; MRP: Maternal recognition period; PBMC: Peripheral blood mononuclear cell; PBS: Phosphate buffered saline; BSA: Bovine serum albumin; P₄: Progesterone; FBS: Fetal bovine serum; RT-PCR: Real-time reverse transcription-polymerase chain reaction; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; eIEI cattle: Cattle with extended interestrous interval (≥ 25 d); nIEI cattle: Cattle with normal interestrous interval (< 25 d).

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Authors' contributions

SM carried out quantitative real-time RT-PCR assays, P₄ assays, AI, ET, and statistical analysis; participated in the design of the study; and drafted the manuscript. TK performed quantitative real-time RT-PCR assays and participated in the design of the study. SK carried out AI and ET and participated in the design of the study. KK performed administration of IFNT into the uterus, recovery of embryos, AI, and ET; participated in the design

of the study; and helped to draft the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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References

- Hernandez-Ledezma JJ, Sikes JD, Murphy CN, Watson AJ, Schultz GA, Roberts RM: Expression of bovine trophoblast interferon in conceptuses derived by in vitro techniques. *Biol Reprod* 1992, **47**(3):374-380.
- Garrett JE, Geisert RD, Zavy MT, Morgan GL: Evidence for maternal regulation of early conceptus growth and development in beef cattle. *J Reprod Fertil* 1988, **84**(2):437-446.
- Mann GE, Fray MD, Lamming GE: Effects of time of progesterone supplementation on embryo development and interferon-tau production in the cow. *Vet J* 2006, **171**(3):500-503.
- Mann GE, Lamming GE: Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. *Reproduction* 2001, **121**(1):175-180.
- Nephew KP, Cardenas H, McClure KE, Ott TL, Bazer FW, Pope WF: Effects of administration of human chorionic gonadotropin or progesterone before maternal recognition of pregnancy on blastocyst development and pregnancy in sheep. *J Anim Sci* 1994, **72**(2):453-458.
- Bartol FF, Roberts RM, Bazer FW, Lewis GS, Godkin JD, Thatcher WW: Characterization of proteins produced in vitro by periattachment bovine conceptuses. *Biol Reprod* 1985, **32**(3):681-693.
- Roberts RM, Cross JC, Leaman DW: Unique features of the trophoblast interferons. *Pharmacol Ther* 1991, **51**(3):329-345.
- Hansen TR, Kazemi M, Keisler DH, Malathy PV, Imakawa K, Roberts RM: Complex binding of the embryonic interferon, ovine trophoblast protein-1, to endometrial receptors. *J Interferon Res* 1989, **9**(2):215-225.
- Spencer TE, Bazer FW: Ovine interferon tau suppresses transcription of the estrogen receptor and oxytocin receptor genes in the ovine endometrium. *Endocrinology* 1996, **137**(3):1144-1147.
- Spencer TE, Becker WC, George P, Mirando MA, Ogle TF, Bazer FW: Ovine interferon-tau inhibits estrogen receptor up-regulation and estrogen-induced luteolysis in cyclic ewes. *Endocrinology* 1995, **136**(11):4932-4944.
- Thatcher WW, Meyer MD, Danet-Desnoyers G: Maternal recognition of pregnancy. *J Reprod Fertil Suppl* 1995, **49**:15-28.
- Vallet JL, Bazer FW, Fliss MF, Thatcher WW: Effect of ovine conceptus secretory proteins and purified ovine trophoblast protein-1 on interovular interval and plasma concentrations of prostaglandins F-2 α and E and of 13,14-dihydro-15-keto prostaglandin F-2 α in cyclic ewes. *J Reprod Fertil* 1988, **84**(2):493-504.
- Hansen TR, Austin KJ, Perry DJ, Pru JK, Teixeira MG, Johnson GA: Mechanism of action of interferon-tau in the uterus during early pregnancy. *J Reprod Fertil Suppl* 1999, **54**:329-339.
- Roberts RM, Cross JC, Leaman DW: Interferons as hormones of pregnancy. *Endocr Rev* 1992, **13**(3):432-452.
- Spencer TE, Burghardt RC, Johnson GA, Bazer FW: Conceptus signals for establishment and maintenance of pregnancy. *Anim Reprod Sci* 2004, **82**:537-550.
- Han H, Austin KJ, Rempel LA, Hansen TR: Low blood ISG15 mRNA and progesterone levels are predictive of non-pregnant dairy cows. *J Endocrinol* 2006, **191**(2):505-512.
- Yankey SJ, Hicks BA, Carnahan KG, Assiri AM, Sinor SJ, Kodali K, Stellflug JN, Ott TL: Expression of the antiviral protein Mx in peripheral blood mononuclear cells of pregnant and bred, non-pregnant ewes. *J Endocrinol* 2001, **170**(2):R7-R11.
- Gifford CA, Racicot K, Clark DS, Austin KJ, Hansen TR, Lucy MC, Davies CJ, Ott TL: Regulation of interferon-stimulated genes in peripheral blood leukocytes in pregnant and bred, nonpregnant dairy cows. *J Dairy Sci* 2007, **90**(1):274-280.
- Bott RC, Ashley RL, Henkes LE, Antoniazzi AQ, Bruemmer JE, Niswender GD, Bazer FW, Spencer TE, Smirnova NP, Anthony RV, Hansen TR: Uterine vein infusion of interferon tau (IFNT) extends luteal life span in ewes. *Biol Reprod* 2010, **82**(4):725-735.
- Oliveira JF, Henkes LE, Ashley RL, Purcell SH, Smirnova NP, Veeramachaneni DN, Anthony RV, Hansen TR: Expression of interferon (IFN)-stimulated genes in extrauterine tissues during early pregnancy in sheep is the consequence of endocrine IFN-tau release from the uterine vein. *Endocrinology* 2008, **149**(3):1252-1259.
- Green JC, Okamura CS, Poock SE, Lucy MC: Measurement of interferon-tau (IFN-tau) stimulated gene expression in blood leukocytes for pregnancy diagnosis within 18-20d after insemination in dairy cattle. *Anim Reprod Sci* 2010, **121**(1-2):24-33.
- Diskin MG, Sreenan JM: Fertilization and embryonic mortality rates in beef heifers after artificial insemination. *J Reprod Fertil* 1980, **59**(2):463-468.
- Dunne LD, Diskin MG, Sreenan JM: Embryo and foetal loss in beef heifers between day 14 of gestation and full term. *Anim Reprod Sci* 2000, **58**(1-2):39-44.
- Roche JF, Boland MP, McGeady TA: Reproductive wastage following artificial insemination of heifers. *Vet Rec* 1981, **109**(18):401-404.
- Silke V, Diskin MG, Kenny DA, Boland MP, Dillon P, Mee JF, Sreenan JM: Extent, pattern and factors associated with late embryonic loss in dairy cows. *Anim Reprod Sci* 2002, **71**(1-2):1-12.
- Horan B, Mee JF, Rath M, O'Connor P, Dillon P: The effect of strain of Holstein-Friesian cow and feeding system on reproductive performance in seasonal calving milk production systems. *Animal Sci* 2004, **79**:453-467.
- Humbolt P: Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies and sources of embryonic mortality in ruminants. *Theriogenology* 2001, **56**(9):1417-1433.
- Imakawa K, Anthony RV, Kazemi M, Marotti KR, Polites HG, Roberts RM: Interferon-like sequence of ovine trophoblast protein secreted by embryonic trophoblast. *Nature* 1987, **330**(6146):377-379.
- Plante C, Hansen PJ, Martinod S, Siegenthaler B, Thatcher WW, Pollard JW, Leslie MV: Effect of intrauterine and intramuscular administration of recombinant bovine interferon α_1 on luteal lifespan in cattle. *J Dairy Sci* 1989, **72**(7):1859-1865.
- Santos JE, Thatcher WW, Chebel RC, Cerri RL, Galvao KN: The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. *Anim Reprod Sci* 2004, **82**:513-535.
- Diskin MG, Murphy JJ, Sreenan JM: Embryo survival in dairy cows managed under pastoral conditions. *Anim Reprod Sci* 2006, **96**(3-4):297-311.
- Kimura K, Hirako M, Iwata H, Aoki M, Kawaguchi M, Seki M: Successful superovulation of cattle by a single administration of FSH in aluminum hydroxide gel. *Theriogenology* 2007, **68**(4):633-639.
- Stringfellow DA, Seidel SM: *Manual of the International Embryo Transfer Society: A procedural guide and general information for the use of embryo transfer technology emphasizing sanitary procedures*. 3 edition. Savoy, IL: International Embryo Transfer Society; 1998.
- Dochi O, Yamamoto Y, Saga H, Yoshida N, Kano N, Maeda J, Miyata K, Yamauchi A, Tominaga K, Oda Y, Nakashima T, Inohae S: Direct transfer of bovine embryos frozen-thawed in the presence of propylene glycol or ethylene glycol under on-farm conditions in an integrated embryo transfer program. *Theriogenology* 1998, **49**(5):1051-1058.
- Takenouchi N, Oshima K, Shimada K, Takahashi M: The development of a sensitive enzyme immunoassay for the determination of estrone and estradiol-17 β in bovine blood plasma based on the same homologous combination with antiserum and steroid-enzyme conjugate. *J Vet Med Sci* 2004, **66**(11):1315-1321.
- Henricks DM, Hill JR, Dickey JF: Plasma ovarian hormone levels and fertility in beef heifers treated with melengestrol acetate (MGA). *J Anim Sci* 1973, **37**(5):1169-1175.
- Bazer FW, Burghardt RC, Johnson GA, Spencer TE, Wu G: Interferons and progesterone for establishment and maintenance of pregnancy: interactions among novel cell signaling pathways. *Reprod Biol* 2008, **8**(3):179-211.
- Spencer TE, Johnson GA, Bazer FW, Burghardt RC: Fetal-maternal interactions during the establishment of pregnancy in ruminants. *Soc Reprod Fertil Suppl* 2007, **64**:379-396.
- Spencer TE, Johnson GA, Bazer FW, Burghardt RC, Palmarini M: Pregnancy recognition and conceptus implantation in domestic ruminants: roles of progesterone, interferons and endogenous retroviruses. *Reprod Fertil Dev* 2007, **19**(1):65-78.

40. Lenschow DJ, Giannakopoulos NV, Gunn LJ, Johnston C, O'Guin AK, Schmidt RE, Levine B, Virgin HW: **Identification of interferon-stimulated gene 15 as an antiviral molecule during Sindbis virus infection in vivo.** *J Virol* 2005, **79**(22):13974-13983.
41. Nakaya T, Sato M, Hata N, Asagiri M, Suemori H, Noguchi S, Tanaka N, Taniguchi T: **Gene induction pathways mediated by distinct IRFs during viral infection.** *Biochem Biophys Res Commun* 2001, **283**(5):1150-1156.
42. Herath S, Lilly ST, Fischer DP, Williams EJ, Dobson H, Bryant CE, Sheldon IM: **Bacterial lipopolysaccharide induces an endocrine switch from prostaglandin F_{2α} to prostaglandin E₂ in bovine endometrium.** *Endocrinology* 2009, **150**(4):1912-1920.
43. Del Vecchio RP, Matsas DJ, Inzana TJ, Sponenberg DP, Lewis GS: **Effect of intrauterine bacterial infusions and subsequent endometritis on prostaglandin F_{2α} metabolite concentrations in postpartum beef cows.** *J Anim Sci* 1992, **70**(10):3158-3162.

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